Environment Pollution and Climate Change

ISSN: 2573-458X

Vorsek, Environ Pollut Climate Change 2024, 8:2

Research Article Open Access

Pseudomonas Aeruginosa and Bacillus Cereus Isolation and Evaluation as PCB-Degrading Bacteria for Environmental Bioremediation

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Abstract

Traditional techniques for treating polychlorinated biphenyls (PCBs) in environmental matrices, like chemical decontamination, soil encapsulation, high-temperature heating, and thermal desorption, have been expensive and have left environmental footprints behind. Thus, they have to make way for bioremediation methods, which are less expensive and produce environmentally friendly goods.

Keywords: Bioremediation bacteria; Environmental protection; Ecosystem.

Introduction

The study's objective was to determine whether PCB-degrading bacteria from their environmental matrix were suitable for use in environmental bioremediation based on published data. By cultivating ash samples from the combustion chamber of an MP400 infectious waste incinerator at Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana, and composite soil samples from a transformer-servicing site at an electricity substation in Sunyani, Ghana, the bacteria was first isolated. In order to test for thermotolerance, bacteria from soil samples were cultured and then exposed to higher temperatures (55 °C and 65 °C) for 48 hours in an incubator. Sub-culturing growing cultures was followed by a variety of characterization assays for bacteria isolates [1-3].

Methodology

Following the sub-culturing of growing cultures, a variety of assays for characterizing bacteria isolates were conducted on the growing isolates. Among these were biochemical analyses of the bacterial suspensions using the analytical profile index (API 20E) to look at non-fastidious bacteria and Enterobacteriaceae. The bacterial strains were then subjected to MALDI-ToF-MS analyses in order to profile and image their proteins [4].

It was necessary to take a molecular step, which involved amplifying and highlighting particular regions of the bacterial DNA extracts using the traditional polymerase chain reaction (PCR) technique with particular primer pairs. The study's findings demonstrated a verified sample location in Ghana where microorganisms that can break down polychlorinated biphenyls (PCBs) can be harvested. Bacillus cereus and Pseudomonas aeruginosa were identified as potential species by MALDI-ToF-MS analyses. According to published data, the presence of bph genes encoding the 2,3-dioxygenase enzymes in bacteria capable of degrading PCBs was verified by traditional PCR and gel electrophoresis techniques. A novel contribution to environmental microbiology was made when the bacterial suspensions were serially diluted from 10−7 to 10−5, allowing for the counting of distinct colonies. It is promising to grow Pseudomonas and Bacillus species in different environmental media, like a gaseous medium to mineralize gas-phase PCBs from combustion smoke or bottom ash residues to degrade solid-phase PCBs, as confirmed by this study's isolation of these species from a soil affected by transformer oil. It can also be used in PCB-containing waste liquid systems for bioremediation [5-7].

There are reports that oil spills are associated with bacteria that have the ability to scavenge. Particularly, the oils used in electrical transformers leak into the underlying soils and are colonized by bacteria that can break down the chemical compound—polychlorinated biphenyls—found in the oil. Because of the high plastic content, these are also found in products that are burned from medical waste. Certain microorganisms gradually colonize sediments or soils that have been contaminated by these persistent organic pollutants. Compared to bacteria from environments where PCBs are absent or present in trace amounts, those that inhabit PCB-contaminated sites are better suited for the de-chlorination or degradation of PCBs. This is thought to be the outcome of environmental selections, where native species are strongly selected against by these xenobiotic compounds in the environment [8, 9].

Accordingly, the soils near leaking transformers may contain bacteria that facilitate PCB degradation. Additionally, since hospital incinerator bottom ash is a combustion byproduct of medical waste with a high plastic content, it can be a favorable environment for microbes that break down PCBs . Because medical wastes that are burned have a high plastic content (PVC), the ash residue from these burns is PCB-rich, which makes it an ideal environment for bacterial colonies that can break down PCBs and withstand high temperatures found in flue gas environments.

Research has attempted to identify and utilize bacteria capable of breaking down or dechlorinating PCBs in sediment or marine environments. Step-by-step, more lipophilic, chlorinated PCB congeners are converted to less lipophilic, less chlorinated congeners during the de-chlorination process. Higher-chlorinated PCBs are electron acceptors that degrade in anaerobic environments, whereas lower-chlorinated PCBs are electron donors that break down in aerobic environments. Different sets of bacteria are involved in this instance, and the conditions vary. In the lab, bacterial colonies that are capable

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Received: 01-Mar-2024, Manuscript No: EPCC-24-130620, **Editor Assigned:** 04-Mar-2024, pre QC No: EPCC-24-130620 (PQ), **Reviewed:** 18-Mar-2024, QC No: EPCC-24-130620, **Revised:** 20-Mar-2024, Manuscript No: EPCC-24-130620 (R), **Published:** 27-Mar-2024, DOI: 10.4172/2573-458X.1000386

Citation: Vorsek A (2024) Pseudomonas Aeruginosa and Bacillus Cereus Isolation and Evaluation as PCB-Degrading Bacteria for Environmental Bioremediation. Environ Pollut Climate Change 8: 386.

of breaking down the lower chlorinated biphenyls under aerobic conditions have been identified. They consist of gram-positive bacteria as well as some classes of gram-negative proteobacteria, including Pseudomonas, Burkholderia, and Sphingomonas.

Only a small number of these bacterial strains, like Rhodococcus globerulus P6 and Burkholderia sp. LB400, are able to mineralize or degrade both phenyl rings, but the majority of them are capable of degrading a wide range of PCB congeners. Two general pathways for PCB biodegradation—aerobic metabolism through reductive dehalogenation and aerobic metabolism via co-metabolism—were also validated by the results of another study [10]. Lower chlorinated biphenyls can be mineralized into their metabolites—chloride, carbon dioxide, water, and biomass—by the aerobic biodegradation of PCBs. When appropriate aerobic microorganisms release 2,3-dioxygenases, the PCBs are broken down into metabolites and then into chlorobenzoic acid. In order to obtain bacteria for use in soil bioremediation, wastewater treatment, flue gas treatment, and other environmental applications, this study collected samples from bottom ash from incinerators and soils contaminated with transformer oils.

Results

The traditional polymerase chain reaction (PCR) thermal cycler (ProFlex Thermocycler, Applied Biosystems, USA) was used to amplify DNA. For the DNA amplification, particular bph primer pairs were utilized, including bphB B1 and bphB B2, bphC F and bphC R, and bphA1 F and bphA1 R. 50 µl was the total volume of the PCR reaction. Approximately 60 ng of extracted bacterial DNA were mixed with 1 µl of Bestar Taq DNA polymerase (2.5 U/µl), 5 µl of deoxynucleoside triphosphates (dNTPs, 2 mM each), 5 µl of 10X Bestar Taq buffer, and 1 µl of each primer (10 µM) in the PCR mixture. With 35 cycles of touchdown PCR denaturation at 94 °C for 30 s and annealing at 60 °C, the thermal cycling program was set at 94 °C for 5 min.

Discussion

Positive test results are indicated by the color codes in Table S1 for the different tests. Transformer soil suspensions used in the assay included 10−2 dilutions (TS 21 and TS 22) and serially diluted 10−1 (TS 11 and TS 12) suspensions in addition to undiluted suspensions (TS 01 and TS 02). Three of the 22 tests that were performed produced positive findings for each of the six bacterial suspensions or dilutions. These included the gelatin liquefaction test (GEL), the arginine dihydrolase test (ADH), and the ortho-nitrophenyl beta-D-galactopyranoside test (ONPG). Therefore, it was determined that all six bacterial suspensions contained the enzymes beta-D-galactosidase, also known as lactase; arginine dihydrolase, which used arginine as a carbon and energy source for bacterial growth; and gelatinase, also known as protease.

The gelatin liquefaction test produced a positive black result, indicating that the bacterial samples' gelatinase (protease) hydrolyzed the gelatin to produce amino acids. On the other hand, the H2S production test (H2S), the urease utilization test (URE), the tryptophan deaminase (TDA), the oxidase (OXI), and the catalase test (CAT) all returned negative results for all six suspensions. Proteus, Ureaplasma, Nocardia, Cryptococcus, Helicobacter, Klebsiella, S. saprophyticus, and S. epidermidis were likely absent, according to these four results. Furthermore, they proposed that the bacteria were not strictly anaerobes. For the bacterial suspensions, the remaining 15 tests produced a range of results. These included the following: glucose fermentation test (GLU), mannose or hexose sugar fermentation test (MAN), isositol fermentation test (INO), sorbitol or alcohol sugar fermentation test (SOR), rhamnose or methyl pentose sugar fermentation test, tryptophan utilization test (Kovac's reagent), and Voges Proskauer test (Barrit's reagent + creatine).

Conclusion

The goal of this study was to collect bacteria from a soil site contaminated by leaking transformer oils and bottom ash from an incinerator. Growth was only observed in inoculums prepared from the contaminated soil site, according to cultural analyses conducted on the two sample sets using basal salt media. Bacilli that were both gram-positive and gram-negative, including species like Bacillus sp., were discovered through additional subculturing, gram staining, and biochemical analyses of the developing cultures. The isolates underwent additional plating and were subjected to MALDI-ToF-MS strain analyses, which identified putative species like Bacillus cereus and Pseudomonas aeruginosa. The presence of bph genes encoding the 2,3-dioxygenase enzymes in bacteria with PCB-degrading ability, which are also established in the literature, was confirmed by conventional PCR and DNA documentation via gel electrophoresis.

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